Reference: *Biol. Bull.* **204:** 126–137. (April 2003)

Role of Maxilla 2 and Its Setae During Feeding in the Shrimp *Palaemon adspersus* (Crustacea: Decapoda)

A. GARM[1,*], E. HALLBERG[2], AND J. T. HØEG[1]

[1]*Department of Zoomorphology, Zoological Institute, University of Copenhagen, Universitetsparken 15, 2100 Copenhagen, Denmark; and* [2]*Department of Cell and Organism Biology, University of Lund, Helgolandsvegen 17, Lund, Sweden*

Abstract. The movements of the basis of maxilla 2 in *Palaemon adspersus* were examined using macro-video recordings, and the morphology of its setae was examined using both scanning and transmission electron microscopy. The basis of maxilla 2 performs stereotypical movements in the latero-medial plane and gently touches the food with a frequency of 3–5 Hz. The medial rim of the basis of maxilla 2 carries three types of seta. Type 1 is serrate, type 2 and 3 are serrulate, and type 2 has a prominent terminal pore. Type 2 is innervated by 18–25 sensory cells whose cilia protrude through the terminal pore and are in direct contact with the external environment. The structure of type 2 setae indicates that they are mainly gustatory, although still bimodal due to their innervation by presumed chemosensory and mechanosensory neurons. Distally, the three types of setae have a complex arrangement of the cuticle involving water-filled canals, which may serve to improve flexibility. Type 1 and 3 setae have fewer sensory cells (4–9) but probably also have a bimodal sensory function. The function of type 1 setae is probably to protect type 2 setae, while type 3 setae might serve to groom the ventral side of the basis of maxilla 1.

Introduction

The decapod mouth apparatus comprises six pairs of limbs, which have as many as 40 parts capable of independent movement (Garm and Høeg, 2001). The parts, which are arranged bilaterally in pairs, have different functions such as collecting, holding, moving, tearing, biting, and adjusting the position of food; creating and directing water currents; and grooming (Robberts, 1968; Kunze and Anderson, 1979; Schembri, 1982; Stemhuis *et al.*, 1998; Garm and Høeg, 2001). These functions depend on the position, movement, and gross morphology of the mouthpart in question, and especially on its setation (Stemhuis *et al.*, 1998; Coelho *et al.*, 2000; Garm and Høeg, 2001). The mouthpart functions just listed are all mechanical in nature; however, like most other crustacean setae, those found on the mouthparts are also part of the sensory system (Paffenhöfer and Loyd, 2000), being either mechanosensory, chemosensory, or both (bimodal). The external morphology of the mouthpart setae of decapods is highly diverse (Schembri, 1982; Lavalli and Factor, 1992; Stemhuis *et al.*, 1998; Johnston, 1999; Garm and Høeg, 2000), which indicates that they have a wide range of both mechanical and sensory functions. A rather good correlation between morphology and function has been demonstrated in insect sensilla (Steinbrecht, 1997, 1998; Keil, 1998); but in crustaceans, only the aesthetasc type of seta is understood in detail (Hallberg *et al.*, 1992, 1997; Steullet and Derby, 1997; Derby, 2000; Steullet *et al.*, 2000a, b). Almost nothing is known about the sensory properties of mouthpart setae except for a few studies on copepods (Paffenhöfer and Loyd, 1999, 2000). Many of the mechanical functions of mouthpart setae are understood, but little is known about their sensory functions, such as when they are used during food handling and what they are sensing.

In decapods, maxilla 2 is responsible for ventilating the gills *via* the scaphognathite (gill bailer), and it therefore moves more-or-less constantly and in a stereotypical way (Garm and Høeg, 2001). This must have a limiting effect on the mechanical functions of this mouthpart during food handling, but it might be suitable for chemosensation of food objects held by the other mouthparts. The constant movement would provide the animal with discrete samples of sensory input, which seems to be important for stimulus

Received 30 August 2002; accepted 2 December 2002.

*To whom correspondence should be addressed. E-mail: Algarm@zi.ku.dk

processing by some chemosensory setae (Goldman and Koehl, 2001). In the squat lobsters *Munida sarsi* and *M. tenuimana,* external morphology correlated with behavior suggests that setae on the medial rim of the basis of maxilla 2 have gustatory properties (Garm and Høeg, 2001). When squat lobsters are sorting the sediment in search of edible items, these setae probe sediment particles frequently (3–4 Hz) just before they are rejected; almost no particles are rejected before they reach maxilla 2. These setae therefore seem to play an important role in deciding whether to eat or reject a food object.

Palaemon adspersus is an omnivorous species of caridean shrimp with a reduced maxilla 2 that lacks the coxal endite (Boas, 1880). This morphology indicates a reduced mechanical function of this limb, making the species especially suited for studying the sensory functions. In this paper, we examine the role of maxilla 2 and its setae during feeding in *P. adspersus*.

Materials and Methods

Video recordings

Adult male and female specimens of *Palaemon adspersus* (carapace lengths 14–20 mm) were obtained from Øresund in Denmark and kept in a 200-l aquarium at Danmarks Akvarium in Copenhagen. Behavioral observations of feeding shrimps were made in 25-l aquaria. Both systems had running seawater at 12 °C. The animals were starved for 3–5 d, then fed mussels, fish meat, live and dead artemia, krill, squid, algal tissue, and muddy sediment; they were then videotaped. During recordings, the carapace was attached to a thin iron bar, which could be moved in all three planes ensuring that position and angle of observation could be manipulated. The animals had a steel nut glued to their carapace with cyanoacrylate glue, and the iron bar was screwed into the nut. A SONY DXC 950P color (Y/C) 3CCD camera equipped with a Micronikkor 105-mm macro lens placed outside the aquarium enabled us to resolve structures about 5 μm wide. Recordings were made on PAL super VHS. Light was obtained from a 120-W bulb. Representative images of mouthpart movements were grabbed with a time resolution of 0.02 s (50 fields/s) using the frame grabber card DVRaptor from Canopus, then imported into CorelDraw 10.0, with a resolution of 720 × 564 pixels. We outlined the involved mouthparts and used the outlines for the serial drawings in Figure 2, which therefore accurately reflect the positions and movements of mouthparts in the video sequences.

Light and scanning electron microscopy

Specimens from the behavioral study were dissected and fixed in 2% formalin. For SEM, the mouthparts were cleaned with a sonicator and manually with a beaver-hair brush. A standard dissection microscope with a camera lucida was used for creating the drawing in Figure 1. SEM preparation followed standard procedures, which did not include using osmium tetraoxide. The micrographs were taken on a JEOL 840 scanning electron microscope and stored digitally with a resolution of 1262 × 1616 pixels using the program SEMafore (JEOL).

Transmission electron microscopy

Clean specimens were obtained by maintaining them in clean artificial seawater for 2 d without feeding. The specimens were anesthetized on ice, dissected, and fixed in cold 2.5% glutaraldehyde and 2% paraformaldehyde in 0.2 *M* sodium cacodylate buffer (pH 7.2) for 3 days. The basis of maxilla 2 was dissected from the rest of the limb to shorten the diffusion distance for the fixative. The specimens were postfixed in 1% osmium tetraoxide for 1 h at room temperature, dehydrated in an ethanol series and acetone, and embedded in Epon resin. Ultrathin sections about 50 nm thick were cut on a Leica UCT ultramicrotome, placed on single-slot grids, and stained with uranyl acetate for 20 min at 60 °C and lead citrate for 4 min at 20 °C. The sections were viewed in a JEOL 1230 transmission electron microscope, and digital pictures with a resolution of 1024 × 1024 pixels were taken using a GATAN 791 Multiscan camera.

Results

Movements

As in all other decapods, the mouth apparatus of *Palaemon adspersus* consists of maxillipeds 1–3 (Mxp1–3), maxillae 1–2 (Mx1–2), mandible, paragnath (not visible in Fig. 1), and labrum (La) (Fig. 1). The endopods of Mxp2–3 are elongate 5-segmented appendages with a high degree of freedom of movement, and Mxp1 and Mx1–2 are flattened and arranged in dorso-ventral layers below the mandible. Mx2 is narrowly placed between Mxp1 and Mx1 and can therefore move rather freely in the medio-lateral plane but has restricted movement in the dorso-ventral plane. When handling food, Mx2 performs medio-lateral movements with a frequency of 3–5 Hz, resulting in the medial edge probing the food (Fig. 2). These movements are stereotypical and do not depend on food type. The size of the food object determines the amplitude of the movements. Mx2 can move to some degree in the dorso-ventral plane and thereby touch the food object in different places without moving it. The left and right Mx2 normally alternate in their movements. When the right Mx2 Bas1 touches the food object, the left Mx2 Bas1 is in a lateral position and starts moving medially (Fig. 2B). After the left Mx2 Bas1 has made contact with the food object, the right Mx2 Bas1 starts moving laterally (Fig. 2C). When in lateral position, the left Mx2 Bas1 moves laterally, and the process starts again (Fig.

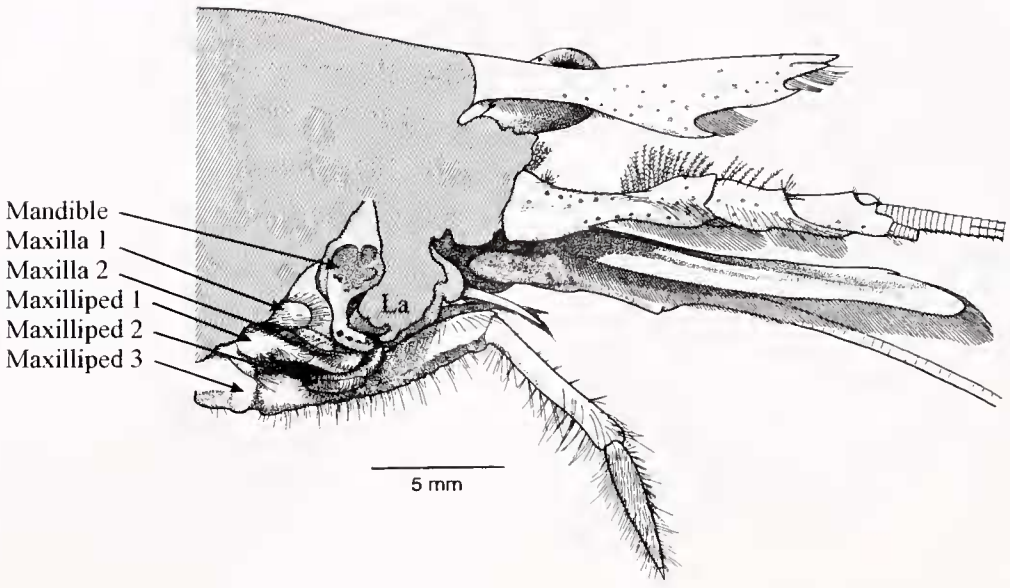

Figure 1. Drawing of the anterior part of *Palaemon adspersus* cut in the medial plane and viewed from the medial side. Striated area indicates cut surface. Position of mouthparts represents the position in an animal not handling any food objects. La = labrum.

2D–F). These movements are made with very few pauses, and Mx2 was never seen to hold the food object. There is a tendency, though, for Mx2 to stop in the lateral position when the mandibles are performing a bite. During the movements, the dorsalmost setae scrape across the ventral side of Mx1 Bas (not shown in Fig. 2 for the sake of simplicity).

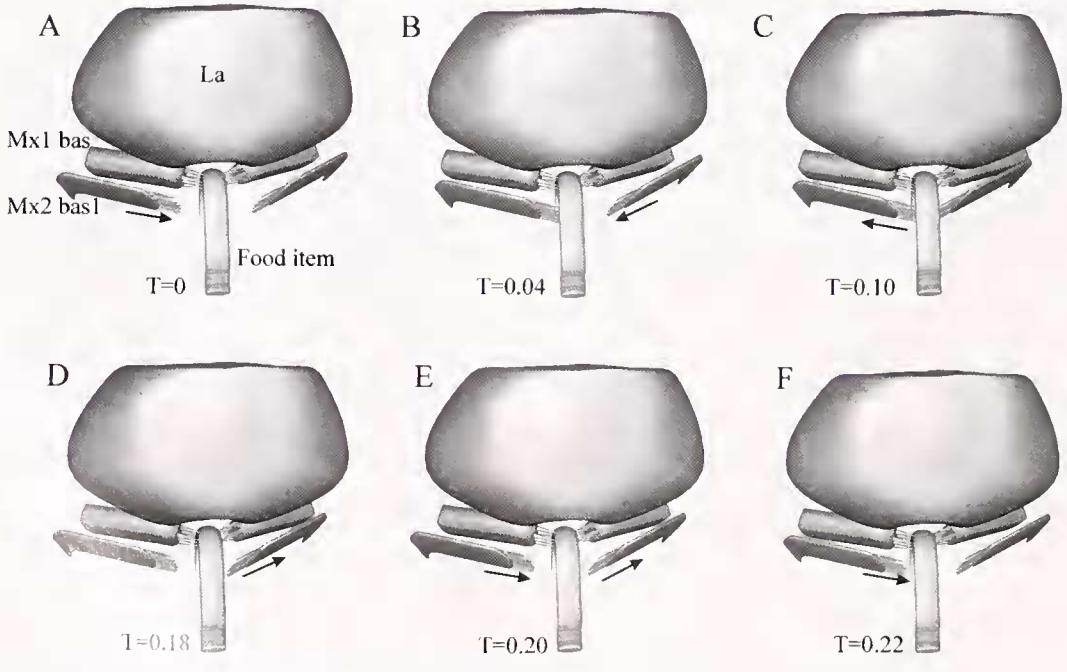

Figure 2. Schematic drawings of latero-medial movements of basis 1 of maxilla 2 during food handling (anterio-ventral view). For simplicity, only the labrum (La), the basis of maxilla 1 (Mx1 Bas), and basis 1 of maxilla 2 (Mx2 Bas1) with type 2 setae are drawn. Arrows indicate direction of movements, and T = time in seconds. (A) Food is held just ventral to La by Mx1 Bas. Mx2 Bas1 are in lateral position, and right Mx2 Bas1 moves medially. (B) Right Mx2 Bas1 in the medial position touches the food, and left Mx2 Bas1 starts moving medially. (C) When left Mx2 Bas1 touches food in medial position, right Mx2 Bas1 moves laterally. (D) When right Mx2 Bas1 is in lateral position, left Mx2 Bas1 moves laterally. (E) Just before left Mx2 Bas1 is in lateral position, right Mx2 Bas1 starts moving medially. (F) A new cycle of movements begins.

External morphology

Mx2 is composed of four parts: the large scaphognathite, a reduced endopod, a very reduced coxa, and a basis (Fig. 3A). The coxa has no endite, but the endite of the basis is well developed and divided into two parts (Fig. 3B, Bas1–2). The medial rim of Bas1, along with the distal part of the medial rim of Bas2, contacts the food objects. This area bears three types of setae arranged in more or less separate rows (Fig. 3C). The terminology in the following descriptions is adapted from the setal classification system proposed by Lavalli and Factor (1992).

Type 1 setae are serrate (setae having denticles in two or more rows) and are the longest (250–350 μm) and most robust of the three types. They form the ventralmost row of 15–18 setae. The seta is slightly curved, tapering distally into a pointed tip, which points dorso-anteriorly (Fig. 4A, B). It lacks terminal and subterminal pores (Fig. 4B). The part of the shaft distal to the annulus carries outgrowths. On one side, they are arranged in two rows angled 120 ° to each other and extend to the tip except for the last 5–10 μm. The rows start as about 10-μm-long setules (articulated with the shaft), gradually changing into 7- to 8-μm-long denticles (not articulated with the shaft) in the distal part of the rows. The denticles get smaller towards the tip. Randomly distributed setules lying flat against the shaft and pointing distally are found opposite the two rows. Proximally, they are 10 to 12-μm long, decreasing to 7–8 μm distally. They terminate 20–30 μm from the tip. The sockets of type 1 setae are reduced and have an intracuticular articulation (Fig. 4A).

Type 2 setae are highly modified, serrulate setae (setae with small elongate or scale-like serrate setules) found in the middle area of Mx2 Bas1+2, but they are not arranged in strict rows (Fig. 3C). They are the most numerous type, with a total of 50–60 on Bas1 + 2. They are slender, a little shorter than type 1 (150–200 μm), and bent slightly into the shape of an S (Fig. 5A). The distal third carries scale-like setules of about 5 μm in length and arranged in three rows. Proximally, the setules lay flat against the shaft, and their tips touch the base of the next setule. More distally on the shaft, the setules are more densely packed so that near the tip they overlap in two to three layers (Fig. 5D). But here they are smaller, reduced to about 1–2 μm in length. The very tip of the seta is a tube, 2 μm long and 1 μm wide, that points dorso-posteriorly and has a prominent terminal pore about 0.5 μm in diameter (Fig. 5B, C). The socket of type 2 setae has an intracuticular articulation and a well-devel-

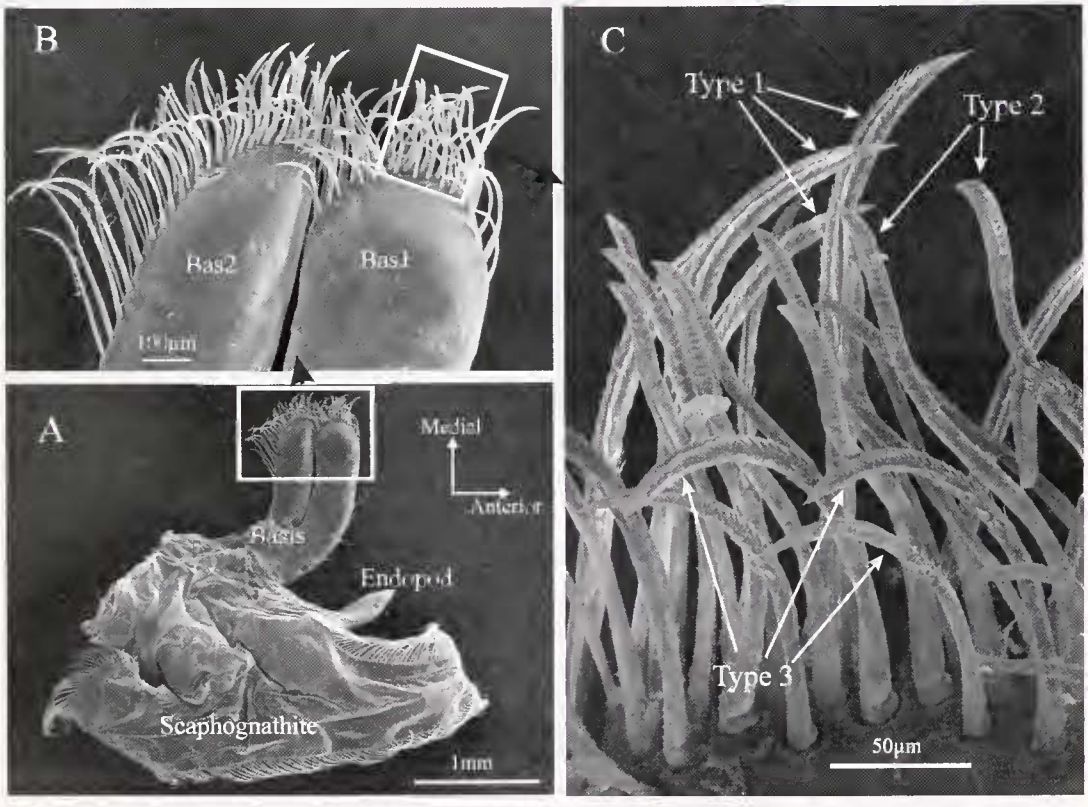

Figure 3. Scanning electron micrographs of maxilla 2 (dorsal view). (A) Whole Mx2 composed of four parts: Scaphognathite (gill bailer), endopod, a divided basis, and coxa (reduced and not visible). (B) Enlargement of framed area in A showing the medial rim of basis 1 and 2 (Bas1, Bas2). Note the change in setation on Bas 2 when moving posteriorly. (C) Enlargement of the framed area in B showing the arrangement of the three types of setae found on the medial rim of Bas1 and Bas2.

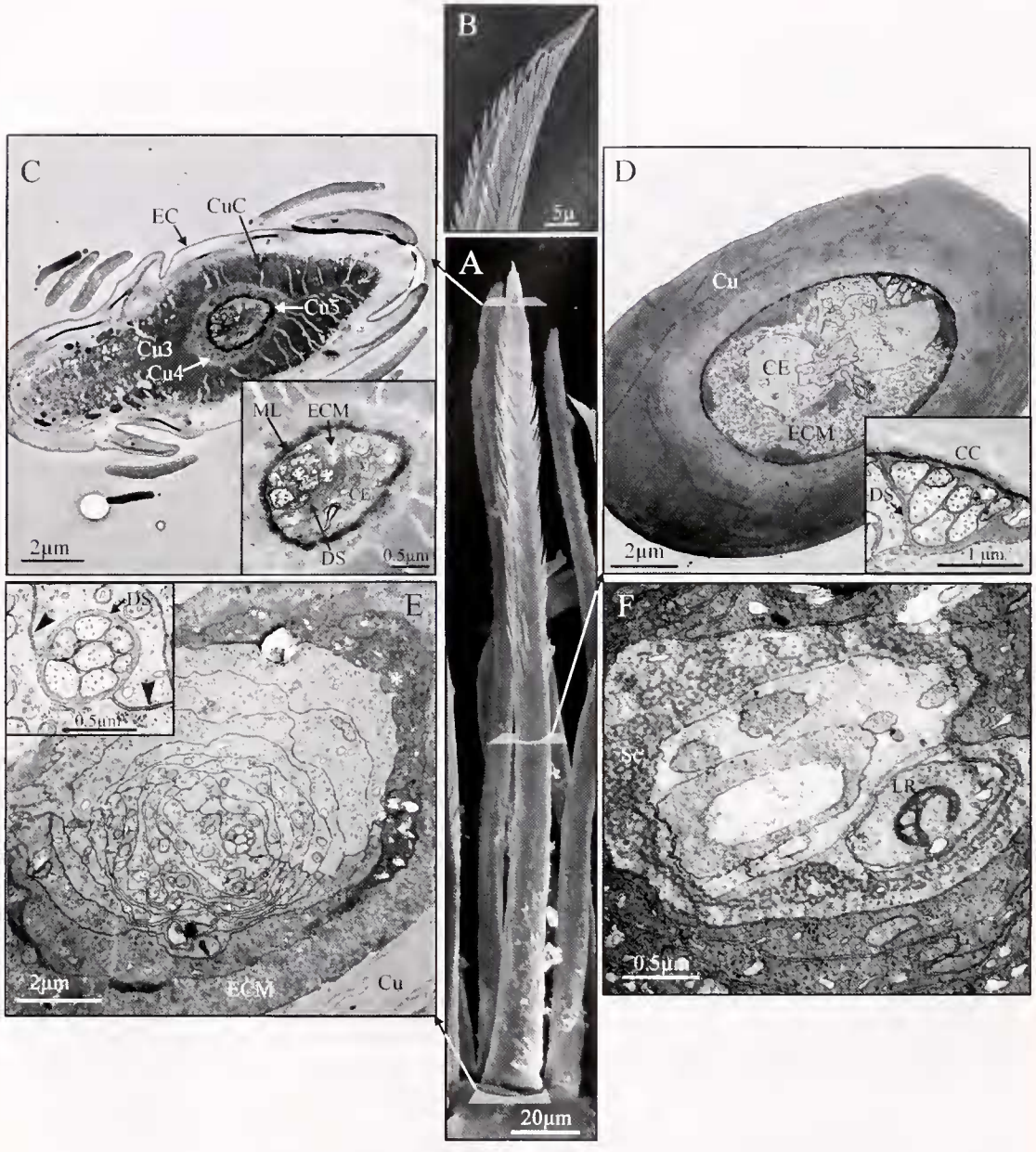

Figure 4. Type 1 seta. (A) Scanning electron micrograph of type 1 seta, which is the longest and most robust seta on Mx2 Bas1. Planes indicate approximate area of transmission electron micrographs. (B) Close-up of pointed tip without pore. (C) Transmission electron micrograph of semi cross-section *ca.* 20 µm from the tip. Cuticle is divided into six distinct layers (EC, Cu2–Cu5, ML). Insert = close-up of lumen containing ciliary compartment (CC, 7 modified cilia) enclosed by a dendritic sheet (DS), one cell extension (CE), and a sparse extracellular matrix (ECM). (D) Cross-section in middle region of seta. Cuticle layers 2 and 3 have fused (Cu). Lumen contains a ciliary compartment with a prominent dendritic sheet (insert), eight cell extensions, and a large amount of extracellular matrix. Insert = close-up of CC. (E) Cross-section just below the base of the seta. Cilia are enclosed by more than 20 sheet cells. Outermost sheet cells are very electron dense and vacuolated (asterisk). Extracellular matrix encircles sheet cell. Insert = close-up of ciliary compartment. Cilia are enclosed by a dendritic sheet, and the sheet cells are connected by septate junctions (arrowheads). (F) Cross-section *ca.* 50 µm below E, showing a large rootlet (LR) in one of the sensory cells. In this area, the innermost sheet cell contains a scolopale (Sc).

oped membranous socket area, which makes the seta rather flexible.

Type 3 setae are serrulate and found in a dorsal row containing about 30 setae (Fig. 3C). It is the shortest of the three types (100–150 µm), is slender, and curves dorsally. The distal half has small setules (5–10 µm) arranged in four

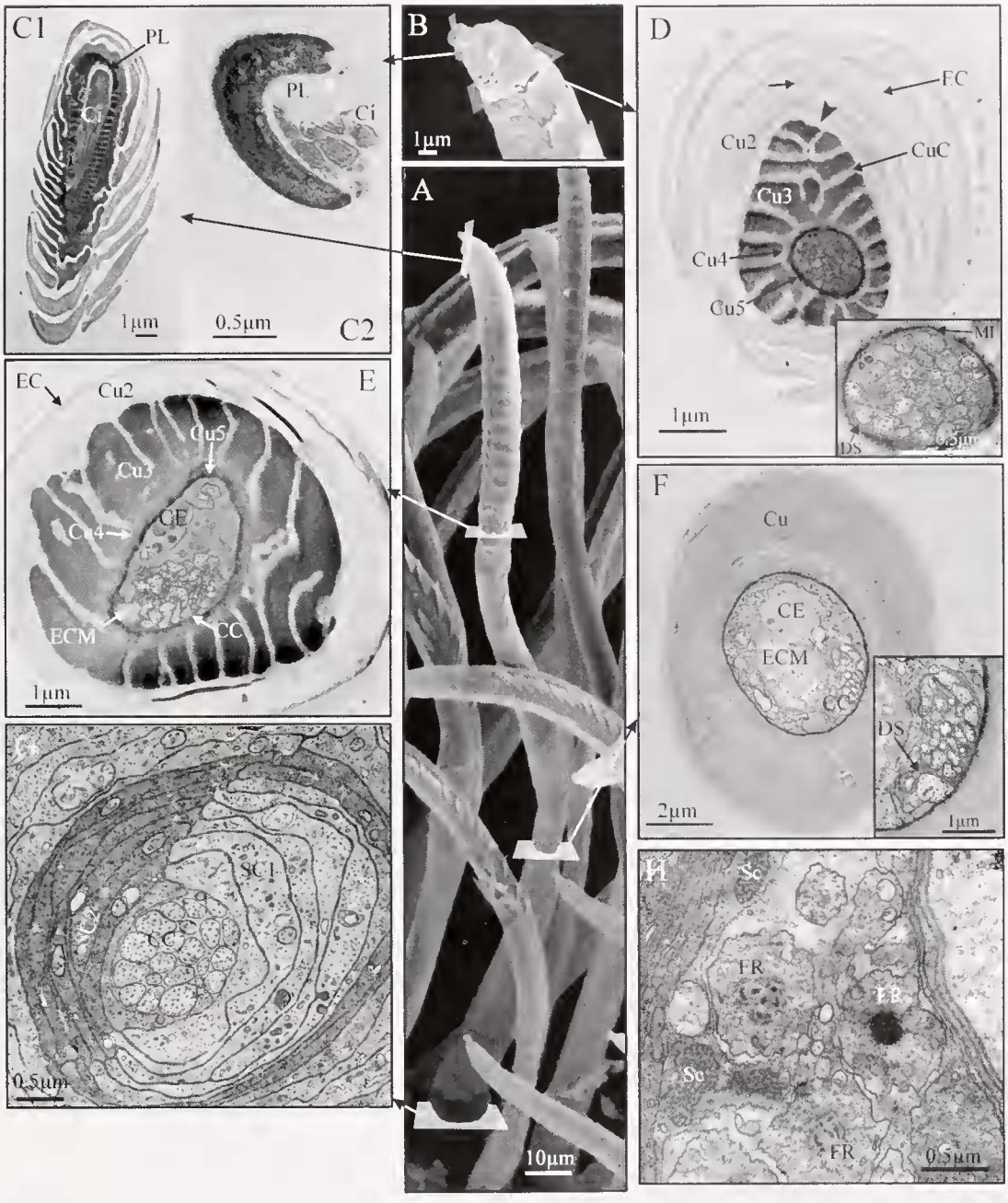

Figure 5. Type 2 seta. (A) Scanning electron micrograph of type 2 seta. Planes indicate approximate area of transmission electron micrographs. (B) Close-up of tip showing prominent terminal pore at the end of a tubular extension. (C1) Longitudinal section of tip showing sensory cilia (Ci) lying in pore lumen (PL). Note that fourth and fifth cuticle layers are missing. (C2) Cross-section of pore showing Ci protruding through pore. (D) Cross-section *ca.* 5 µm from tip. Lumen is completely filled with 30 Ci. Cuticle divided in six layers (EC, Cu2–Cu5, MI). Cu3 with very prominent cuticular canals (CuC). Insert = close-up of lumen. (E) Cross-section in the proximal end of setulate region. Cuticle in five distinct layers and lumen with two cellular extensions (CE), sparse extracellular matrix (ECM), and a ciliary compartment (CC). (F) Cross-section in middle part of nonserrulate area. Cuticular layers are more or less fused. Lumen contains 5 cellular extensions, a large amount of extracellular matrix, and a ciliary compartment enclosed by a dendritic sheet (insert, DS). Insert = close up of CC. (G) Cross-section just below the base of the seta. A ciliary compartment with 24 modified cilia encircled by two types of semicircular sheet cells (SC1, SC2); no dendritic sheet in this area. (H) Approximately 40 µm after G, showing two types of ciliary rootlets and a reduced scolopale. FR = fragmented rootlet, LR = solid rootlet, Sc = scolopale.

rows terminating 5 μm from the setal tip, which lacks pores (Fig. 6A, B). The setules are serrate and decrease in size towards the tip of the seta. The socket forms an intracuticular articulation, and the membranous area is prominent, which adds flexibility to the seta.

Internal morphology

Type 1 setae (Fig. 4) are innervated by five to nine sensory cells (mean = 7.5, n = 10), each of which gives rise to one modified cilium (Fig. 4D). The unbranched cilia continue to the tip of the seta and contain relatively few strands of microtubules (Fig. 4C). Two of the sensory cells contain a large rootlet in their distal end of the cell proper (Fig. 4F, LR), while the other cells have small fragmented rootlets. The numerous sheet cells (>20) tend to lie in semicircles around the bundle of modified cilia (Fig. 4E) and fall into two types. The majority are rather electron-lucent, but two to four of them are electron-dense and contain many vacuoles (Fig. 4E, asterisk). The innermost sheet cell contains a scolopale (Fig. 4F) and forms a dendritic sheet, which encircles the cilia from just below the basal part of the seta to the distalmost 20 μm. In the region of the scolopale, the innermost sheet cell makes a projection that surrounds one of the sensory cells with a large rootlet (Fig. 4F). The extracellular matrix within the lumen of the dendritic sheet is electron-dense. The next five to eight sheet cells send projections into the lumen of the setal shaft (Fig. 4D), and one of these projections continues to within at least 20 μm below the tip (Fig. 4C). We could not identify which sheet cell makes this projection. An extracellular matrix encircles both the ciliary compartment and the cell projections in the setal lumen. The extracellular matrix is most prominent at the base and barely detectable near the tip (Fig. 4C). It contains both electron-dense and electron-lucent areas. The cuticle of the seta is rather thick, and in the distal part it is divided into six distinct layers (Fig. 4C). The outermost thin epicuticle and the *ca.* 1-μm-thick, electron-lucent second layer are the only ones present in the outgrowths of the seta (Fig. 4C). The third layer is electron-dense and contains radially projecting canals that seem to be composed of the same material as the second layer. The cuticle of the convex side of the seta is more granular and without canals. Due to an oblique cross-section of the seta, the third layer varies between 1 and 2 μm in thickness. The fourth layer is about 0.5 μm thick, fibrous, and changes gradually into the fifth layer, which is similarly fibrous but very electron-dense and about 0.1 μm thick. A sixth membranous layer encircles the lumen (Fig. 4C, insert: ML). In the proximal two-thirds of the shaft, the second and third layers have gradually merged to form one homogeneous electron-dense layer 2–4 μm thick (Fig. 4D). The fourth layer has expanded to a thickness of about 1 μm. The fifth layer is not detectable, but the innermost membranous layer remains unchanged.

Type 2 (Fig. 5) is innervated by 18–25 sensory cells, which give rise to 18–25 modified cilia (mean = 23.25, n = 12) (Fig. 5G). Some of these cilia branch, and there are 28–30 ciliary branches in the distal end of the lumen (Fig. 5D, F). The cilia continue all the way to the tip of the seta, where they protrude through the terminal pore, which lies in the extension of the setal lumen (Fig. 5, C1, C2). All the sensory cilia are alike and contain rather few microtubules, in the distal part only 4–10 strands. Two of the sensory cells have a large rootlet; the rest have a small fragmented rootlet. The sensory cells are again surrounded by numerous semicircular sheet cells (>20), which are dimorphic in their

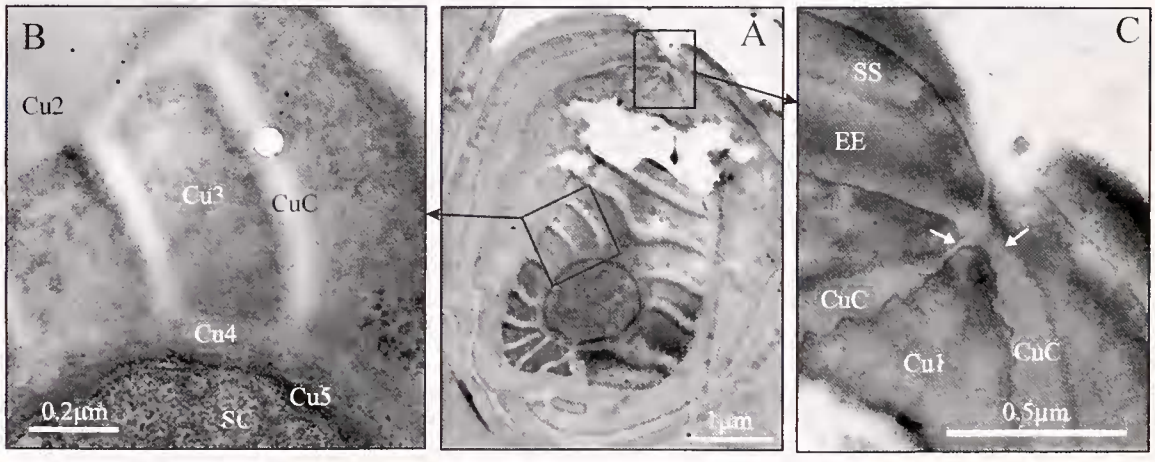

Figure 6. Canal system in the third layer of cuticle of seta type 2. (A) Cross-section of type 2 seta *ca.* 5 μm from the tip. Section is in a rather poor condition because it is the first in a series. (B) Close-up of section in A, showing cuticular canals (CuC) interconnected by circular canal. (C) Close-up of section in A, showing the pores (arrows) where cuticular canals connect with external environment (EE). Cu2–Cu5 = cuticular layers 2–5, SC = sensory cilia, SS = scale-like setule.

electron density and arranged in a variable pattern. About 10 of the sheet cells send projections into the setal lumen. Midway up the setal shaft, the number of cell projections is reduced to three to four, and at the tip only the modified sensory cilia are present within the lumen (Fig. 5D–F). The innermost sheet cell forms the dendritic sheet, which runs from just below the base of the seta all the way to the tip (Fig. 5D, insert) and thus encloses the sensory cilia in a sparse electron-dense extracellular matrix. The innermost sheet cell also contains a rather rudimentary scolopale (Fig. 5H). From the base of the seta, there is also an extracellular matrix in the setal lumen outside the ciliary compartment, which narrows distally and is absent at the tip. The cuticle is of the same thickness and structure as described for type 1 setae, but it is not granular, and the canals in the electron-dense third layer are better developed. The second layer is also thinner in the distal part (Fig. 5D). The tube of the terminal pore appears to be formed from the third layer, but it does not contain any canals (Fig. 5, C1, C2). The canals in type 2 setae are connected to the external environment (Fig. 6). The radial canals are interconnected by a circular canal, which runs on the outside of the third cuticle layer (Fig. 5D, arrowhead; Fig. 6B). This canal in turn is connected to the exterior *via* a pore (Fig. 6C, arrow). These pores are made from infoldings of the epicuticle and second cuticular layer (Fig. 5D, arrow). Unfortunately, the series of sections is not complete, and we are therefore not able to show if the openings are really pores or if they continue and expand further distally.

Type 3 setae (Fig. 7) are innervated by four to nine sensory cells (mean = 6.1, n = 11), each giving rise to one modified cilium (Fig. 7E, F). These cilia continue unbranched to the tip (Fig. 7C). One or two of the sensory cells have a large rootlet; the rest have fragmented rootlets. The type 3 seta has fewer (<20) enveloping cells than the two other types (Fig. 7E). At least six of them send projections into the lumen, and one proceeds to at least 20 μm below the tip (Fig. 7C). The innermost cell forms the dendritic sheet and contains a well-developed scolopale. In the region of the scolopale, the innermost sheet cell encircles one or two of the sensory cells. As in the other types, the dendritic sheet forms the ciliary compartment, which contains the modified cilia and a sparse extracellular matrix. The extracellular matrix in the setal lumen is similar to that found in the two other types. The cuticle is also similar to what is found for type 2, except that the tube is lacking in type 3.

Discussion

We found that maxilla 2 of *Palaemon adspersus* performs stereotypical movements when the animal is handling food items. The medial rims of basis 1 and 2 of maxilla 2 touch the food items gently and were never seen to directly manipulate them. This area of maxilla 2 carries three types of setae, each with different external and internal morphology. Type 1 is serrate, robust, and has 5–9 sensory cells. Type 2 is serrulate, slender, and has 18–25 sensory cells; it also has a terminal pore from which sensory cilia protrude into the external environment. Type 3 is serrulate, short, and possesses four to nine sensory cells. In all three types, the sensory cells fall into two distinct morphological groups. Putative sensory properties of the three types are summarized in Table 1.

Our results on the activity and morphology of maxilla 2 and its setae in *Palaemon adspersus* lead us to conclude that the basis of maxilla 2 collects chemical information from a dense population of gustatory setae before the food item is either eaten or rejected.

The ultrastructure indicates that the three types of setae have distinct and separate functions. Type 1 and type 3 setae are innervated by four to nine sensory cells, one or two of which have a well-developed rootlet in close contact with a scolopale in the innermost sheet cell. A scolopale is an electron-dense structure made of closely packed strands of microtubules bound together with accessory proteins. This indicates that these cells are mechanoreceptors, since it is believed that desmosomal connections between the scolopale and the cilia rootlet are necessary for transduction of the mechanical signal (Schmidt and Gnatzy, 1984; Derby, 1989; Crouau, 2001). The remaining sensory cells have a small fragmented rootlet that is not in contact with the scolopale; they lack dynein arms in the ciliary region, and there are few strands of microtubules in the paraciliary region. This suggests that they are chemoreceptor neurons, but this assumption is based on the absence of mechanoreceptive structures (Schmidt and Gnatzy, 1984; Derby, 1989; Gleeson *et al.*, 1996; Hallberg and Hansson, 1999). We therefore conclude that type 1 and type 3 setae are bimodal sensors capable of detecting both mechanical and chemical stimuli. The existing ultrastructural studies on decapod setae suggest that this is the most common type of sensory innervation (Altner *et al.*, 1983; Schmidt and Gnatzy, 1984; Derby, 1989; Cate and Derby, 2001, 2002). We emphasize that our conclusions are based on behavior and morphology alone, and that no certainty about the sensory properties can be obtained without employing other methods such as electro-physiological recordings from the sensory cells. Although both type 1 and 3 setae are probably bimodal, their size, shape, and arrangement indicate that they have different functions.

The long, robust, and almost straight type 1 setae seem well suited to act as "guard setae," protecting the rather fragile type 2 setae from too much mechanical stress when food objects are being handled. A similar system is found on the first antennae of many decapod crustaceans, including the spiny lobster, *Panulirus argus,* where the aesthetases (fragile chemosensory setae) are protected by long robust guard setae (Steullet *et al.*, 2000b; Cate and Derby, 2001). In the case of the spiny lobster, the ultrastructure of these setae is still unknown, and the protective function is as-

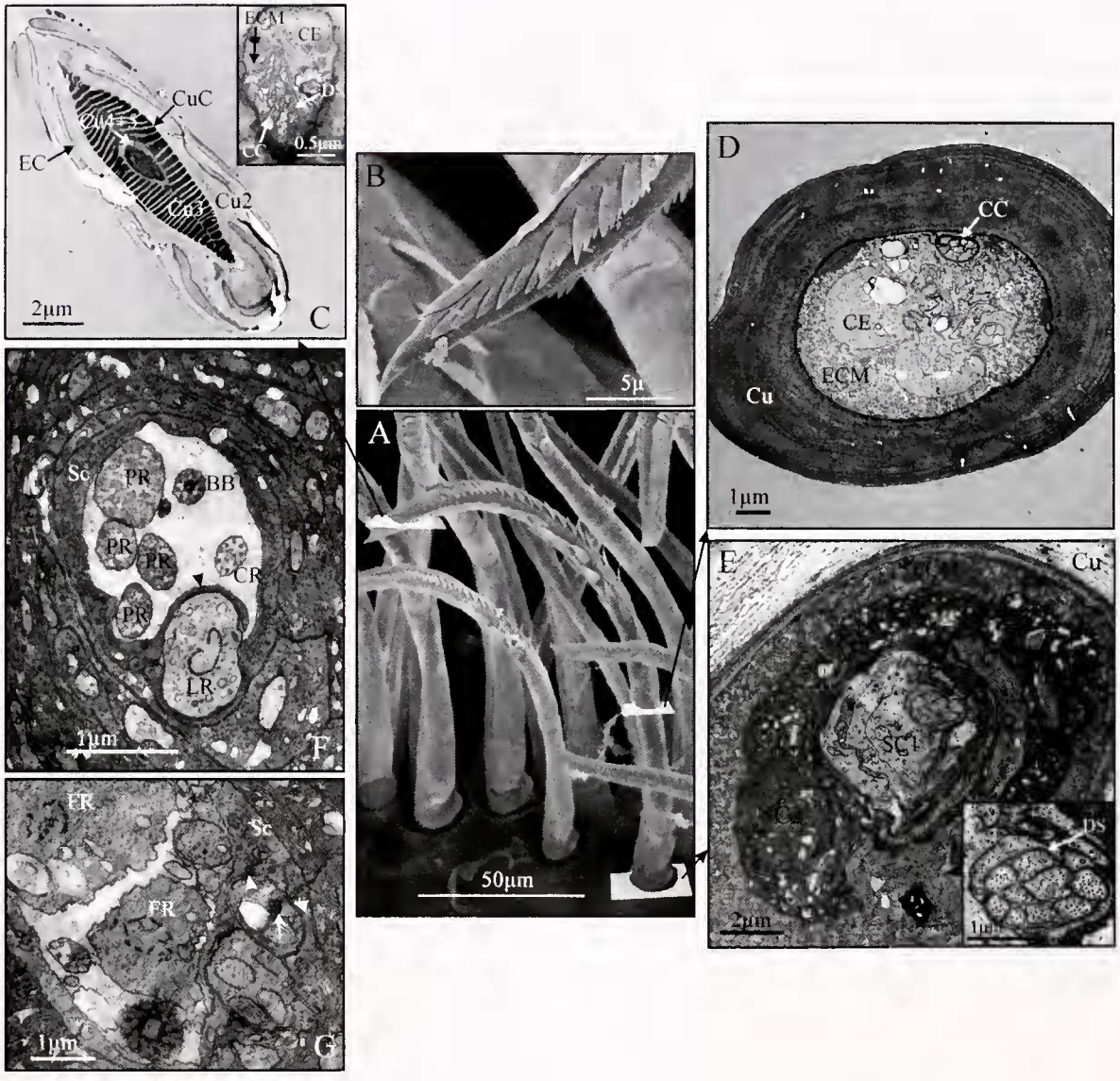

Figure 7. Type 3 seta. (A) Scanning electron micrograph of type 3 seta in dorsal row. Planes indicate approximate area of transmission electron micrographs. (B) Close-up of the tip. (C) Semi cross-section *ca.* 10 µm from tip. The cuticle is divided into six distinct layers (EC, Cu2-Cu5, ML not shown). Cu3 has prominent canals (CuC). Insert = close-up of lumen with ciliary compartment (CC) enclosed by the dendritic sheet (DS), one cell extension (CE), and a sparse extracellular matrix (ECM). (D) Cross-section in nonserrulate part of seta. Cuticle layers 2–5 have fused to form one more or less homogenous layer. Prominent lumen with large amount of extracellular matrix, eight to nine cell extensions, and a ciliary compartment with nine sensory cilia. (E) Cross-section just below the base of the seta. The ciliary compartment is enclosed by a dendritic sheet and 15–20 semicircular sheet cells of two types (SC1, SC2). The sheet cells are surrounded by extracellular matrix. Insert = close-up of ciliary compartment. (F) Cross-section *ca.* 50 µm after E. One cell has a large rootlet (LR); the other cells are sectioned in the region of the basal body (BB) or in the ciliary region (CR) or the paraciliary region (PR). A weak scolopale is seen (Sc). Arrowhead indicates extrusion of sheet cell enclosing sensory cell. (G) Cross section *ca.* 5 µm after F. The putative chemosensory cells contain a fragmented rootlet (FR). The mechanosensory cell is in contact with the scolopale *via* desmosomes (arrowheads). Arrow indicates large ciliary rootlet.

sumed from their arrangement and external morphology. The guard setae on *P. argus* are not serrate but simple (no outgrowths). Most other systems with crustacean mechanosensitive setae studied so far are sensitive to movements of the surrounding water (Wiese, 1976; Heinisch and Wiese, 1987; Derby, 1989); but this does not seem to be the case with the type 1 setae since the socket is reduced, they have no outgrowths suitable to receive water movements, and they are themselves moved all the time. Due to the activity and position of type 1 setae in *Palaemon adspersus*, we

Table 1

Summary of the structures and suggested functions for seta type 1, 2, 3 of Palaemon adspersus

	Type of seta	Length in μm	Pore*	Neurons/cilia	Scolopale*	Modality	Primary function
Type 1	Serrate	250–300	−	4–9	+	Bimodal	Protection
Type 2	Serrulate	200–250	+	18–25	+	Bimodal	Gustation
Type 3	Serrulate	150–200	−	4–9	+	Bimodal	Grooming

* Plus indicates presence; − indicates absence.

believe that they contain mechanoreceptor neurons that function as proprioceptors, providing information on the amount of pressure the animal puts on the food object and thus ensuring that it does not break the setae. The chemosensory function of type 1 setae is not clear but might be gustatory.

Due to their position, curvature, and small size, the serrulate type 3 setae cannot be guard setae. They are more likely used for grooming the ventral side of the basis of maxilla 1, which they scrape every time these mouthparts pass each other. This explanation is consistent with previous studies on decapods in which serrulate setae have been found to be involved in grooming (Martin and Felgenhauer, 1986; Bauer, 1989, 1999; Nickell *et al.*, 1998), and the scale-like setules serve to remove debris.

The type 2 setae are also bimodal sensors, and their primary function is probably gustatory, as indicated by their many chemosensory cells and by the observation that the sensory cilia protrude through the terminal pore. This also indicates that even though type 1 and 3 setae may be chemosensory, the type 2 setae are probably the primary chemosensors of Mx2, and their mechanosensory function may again be proprioceptive.

The type 2 seta is the first decapod seta to be reported in which cilia protrude from a terminal pore. Terminal pores are rather common in decapod setae, but so far they have been studied only with SEM (Altner *et al.*, 1986; Pinn *et al.*, 1999; Garm and Høeg, 2000; Coelho and Rodrigues, 2001). This might fail to reveal protruding cilia, because the treatment during dehydration and critical-point drying may cause shrinkage of the soft tissue, thereby retracting the sensory cilia. Naked sensory cilia of presumed chemosensors of other crustaceans, *Hutchinsoniella macracantha* (Cephalocarida) and *Pachypygus gibber* (Copepoda), have been shown by TEM, and in the former case also by SEM, although the documentation is not of the highest quality (Hipeau-Jacquotte, 1986; Elofsson and Hessler, 1994). In both cases, these setae are suggested to be gustatory, but they differ structurally from what is described here for type 2 setae of *Palaemon adspersus* in that they are small (<30 μm), have a very thin cuticle, and are innervated by only two to three sensory cells. They are also presumed to be unimodal gustatory setae (no mechanosensory function) (Hipeau-Jacquotte, 1986; Elofsson and Hessler, 1994), which has also been suggested for setae of the amphipod *Anonyx lilljeborgi* (Steele and Steele, 1999).

The number and arrangement of the sheet cells resemble earlier reports from decapod setae (Wiese, 1976; Ball and Cowan, 1977; Hallberg *et al.*, 1992). The division into two types of sheet cells might have a functional explanation. In the mysid *Neomysis integer*, the sheet cells are arranged in populations, with each creating a separate part of the cuticle of the setae (Guse, 1980). Our results show a tendency toward an outer population of electron-dense cells and an inner population of electron-lucent cells, the latter sending cell extensions into the lumen of the seta. Although we have no direct proof, we suggest that the electron-dense cells form the basal part of the cuticle of the seta and the electron-lucent cells form the distal part. A projection of the innermost sheet cell that encircles the mechanosensory cells has not been reported before for any crustacean setae. We are not sure of the function of this arrangement, but it could help to anchor the sensory cells and thereby improve the transduction of the mechanosensory signal.

In all three types of setae, the arrangement of the cuticle is extremely complex, with the differentiation throughout the shaft giving the distal part as many as six distinct layers (EC, Cu2–Cu5, ML) and water-filled canals. This could indicate that the distal parts of these setae are experiencing great mechanical stress, which they overcome by having several kinds of cuticle with different mechanical properties. The highly specialized water-filled canals have not been reported before in crustacean setae. One functional explanation could be that some setae involved in handling food objects must be flexible in their distal parts. This means that the cuticle must be able to fold, stretch, or change volume. Having a water-filled canal system gives the tissue the capability of locally changing the volume by moving water. The opening to the exterior also makes it possible to change the volume of the entire canal system; however, the minute size of the opening may limit the speed of this process. As mentioned earlier, cross-sections of the distalmost part of the canal system are missing, and it is possible that the openings expand further distally. Some earlier studies found similar electron-lucent canals in the cuticle (Crouau, 1997; Matsuura and Nishida, 2000; Cate and Derby, 2002) but did not discuss them in detail, and no connection to the exterior was described.

The activity of maxilla 2 in *Palaemon adspersus* is very stereotypic. We never observed the Mx2 to mechanically influence or hold the food; it merely touched it frequently (3–5 Hz). This behavior supports the idea that some of the setae on the medial rims of basis 1 and 2 of maxilla 2 have gustatory properties like those proposed for maxilla 2 of other decapods (Schembri, 1982; Garm and Høeg, 2001). Mechanical functions of maxilla 2 are, however, described for these other decapods. In the anomurans *Munida sarsi* and *M. tenuimana*, the basis of maxilla 2 is additionally used to reorient small food objects (Garm and Høeg, 2001). In the hermit crab *Pagurus rubricatus*, the basis of maxilla 2 assists in filtering sediment before eating (Schembri, 1982). In the case of *Homarus gammarus*, maxilla 2 is described as having mechanical functions, but the observations are not very detailed (Barker and Gibson, 1977). In other decapods for which detailed observations of mouthpart movements have been made, no account is given for the movements of maxilla 2 (Hunt *et al.*, 1992; Stemhuis *et al.*, 1998; Johnston, 1999), but judging from the descriptions of the morphology and feeding behavior in general, the usage is likely to be similar to what we found for *P. adspersus*.

Acknowledgments

The authors gratefully acknowledge the excellent laboratory work done by Rita Wallén, Department of Cell and Organism Biology, University of Lund. We are very grateful to the staff of the Danmarks Akvarium, Copenhagen, who collected and kept the animals for us. Jens T. Høeg acknowledges grants from the Danish National Science Research Council for financial support.

Literature Cited

Altner, H., H. Hatt, and I. Altner. 1986. Structural and functional properties of the mechanoreceptors and chemoreceptors in the anterior oesophageal sensilla of the crayfish, *Astacus astacus*. *Cell Tissue Res.* **244:** 537–547.

Altner, I., H. Hatt, and H. Altner. 1983. Structural properties of bimodal chemo- and mechanosensitive setae on the pereiopod chelae of the crayfish, *Austropotamobius torrentium*. *Cell Tissue Res.* **228:** 357–374.

Ball, E. E., and A. N. Cowan. 1977. Ultrastructure of the antennal sensilla of *Acetes* (Crustacea, Decapoda, Natantia, Sergestidea). *Philos. Trans. R. Soc. Lond. B* **227:** 429–457.

Barker, P., and R. Gibson. 1977. Observations on the feeding mechanism, structure of the gut, and digestive physiology of the European lobster *Homarus gammarus* (L.) (Decapoda: Nephropidea). *J. Exp. Mar. Biol. Ecol.* **26:** 297–324.

Bauer, R. T. 1989. Decapod crustacean grooming: functional morphology, adaptive value, and phylogenetic significance. Pp. 49–74 in *Crustacean Issues 6, Functional Morphology of Feeding and Grooming in Crustacea*, B. Felgenhauer, L. Watling, and A. B. Thistle, eds. A. A. Balkema, Rotterdam.

Bauer, R. T. 1999. Gill-cleaning mechanisms of a dendrobranchiate shrimp, *Rimapenaeus similis* (Decapoda, Penaeidae): description and experimental testing of function. *J. Morphol.* **242:** 125–139.

Boas, J. E. V. 1880. *Studier over Decapodernes Slægtsskabsforhold.*, Bianco Lunds Kgl. Hof-Bogtrykkeri, Copenhagen. Pp. 1–210.

Cate, H. S., and C. D. Derby. 2001. Morphology and distribution of setae on the antennules of the Caribbean spiny lobster *Panulirus argus* reveal new types of bimodal chemo-mechanosensilla. *Cell Tissue Res.* **304:** 439–454.

Cate, H. S., and C. D. Derby. 2002. Ultrastructure and physiology of the hooded sensillum, a bimodal chemo-mechanosensillum of lobsters. *J. Comp. Neurol.* **442:** 293–307.

Coelho, V. R., and S. A. Rodrigues. 2001. Trophic behaviour and functional morphology of the feeding appendages of the laomediid shrimp *Axianassa australis* (Crustacea: Decapoda: Thalassinidea). *J. Mar. Biol. Assoc. UK* **81:** 441–454.

Coelho, V. R., A. B. Williams, and S. A. Rodrigues. 2000. Trophic strategies and functional morphology of feeding appendages, with emphasis on setae of *Upogebia omissa* and *Pomatogebia operculata* (Decapoda: Thalassinidea: Upogebiidae). *Zool. J. Linn. Soc.* **130:** 567–602.

Crouau, Y. 1997. Comparison of crustacean and insect mechanoreceptive setae. *Int. J. Insect Morphol. Embryol.* **26:** 181–190.

Crouau, Y. 2001. Cellules méchanosensorielles des soies des Hexapodes, des Crustacés et des Myriapodes: une comparaison d'un point de vue phylogénétique. *Ann. Soc. Entomol. Fr.* **37:** 233–242.

Derby, C. D. 1989. Physiology of sensory neurons in morphologically identified cuticular sensilla of crustaceans. Pp. 27–48 in *Crustacean Issues 6, Functional Morphology of Feeding and Grooming in Crustacea*, B. Felgenhauer, L. Watling, and A. B. Thistle, eds. A. A. Balkema, Rotterdam.

Derby, C. D. 2000. Learning from spiny lobsters about chemosensory coding of mixtures. *Physiol. Behav.* **69:** 203–209.

Elofsson, R., and R. R. Hessler. 1994. Sensory structures associated with the body cuticle of *Hutchinsoniella macracantha* (Cephalocarida). *J. Crustac. Biol.* **14:** 454–462.

Garm, A., and J. T. Høeg. 2000. Functional mouthpart morphology of the squat lobster *Munida sarsi*, with comparison to other anomurans. *Mar. Biol.* **137:** 123–138.

Garm, A., and J. T. Høeg. 2001. Function and functional groupings of the complex mouth apparatus of the squat lobsters *Munida sarsi* Huus and *M. tenuimana* G. O. Sars (Crustacea: Decapoda). *Biol. Bull.* **200:** 281–297.

Gleeson, R. A., L. M. McDowell, and H. C. Aldrich. 1996. Structure of the aesthetasc (olfactory) sensilla of the blue crab, *Callinectes sapidus*: transformations as a function of salinity. *Cell Tissue Res.* **284:** 279–288.

Goldman, J. A., and M. A. R. Koehl. 2001. Fluid dynamic design of lobster olfactory organs: high speed kinematic analysis of antennule flicking by *Panulirus argus*. *Chem. Senses* **6:** 385–398.

Guse, G. W. 1980. Development of antennal sensilla during moulting in *Neomysis integer* (Leach) (Crustacea, Mysidacea). *Protoplasma* **105:** 53–67.

Hallberg, E., and B. S. Hansson. 1999. Arthropod sensilla: morphology and phylogenetic considerations. *Microsc. Res. Tech.* **47:** 428–439.

Hallberg, E., K. U. I. Johansson, and R. Elofson. 1992. The aesthetasc concept: structural variations of putative olfactory receptor cell complexes in Crustacea. *Microsc. Res. Tech.* **22:** 325–335.

Hallberg, E., K. U. I. Johansson, and R. Wallén. 1997. Olfactory sensilla in crustaceans: morphology, sexual dimorphism, and distribution patterns. *Int. J. Insect Morphol. Embryol.* **26:** 173–180.

Heinisch, P., and K. Wiese. 1987. Sensitivity to movement and vibration of water in the North Sea shrimp *Crangon crangon* (L.). *J. Crustac. Biol.* **7:** 401–413.

Hipeau-Jacquotte, R. 1986. A new cephalic type of presumed sense organ with naked dendritic ends in the atypical male of the parasitic copepod *Pachypygus gibber* (Crustacea). *Cell Tissue Res.* **245:** 29–35.

Hunt, M. J., H. Winsor, and C. G. Alexander. 1992. Feeding by the penaeid prawns: the role of the anterior mouthparts. *J. Exp. Mar. Biol. Ecol.* **160:** 33–46.

Johnston, D. J. 1999. Functional morphology of the mouthparts and alimentary tract of the slipper lobster *Thenus orientalis* (Decapoda: Scyllaridae). *Mar. Freshw. Res.* **50:** 213–223.

Keil, T. A. 1998. The structure of integumental mechanoreceptors. Pp. 385–404 in *Microscopic Anatomy of Invertebrates, Insecta,* F. W. Harrison and M. E. Rice, eds. Wiley-Liss, New York.

Kunze, J., and D. Anderson. 1979. Functional morphology of the mouthparts and gastric mill in the hermit crabs *Clibanarius taenitua* (Milne Edwards), *Clibanarius virescens* (Krauss), *Paguristes squamousus* McCulloch and *Dardanus setifer* (Milne-Edwards) (Anomura: Paguridea). *Aust. J. Mar. Freshw. Res.* **30:** 683–722.

Lavalli, K. L., and J. R. Factor. 1992. Functional morphology of the mouthparts of juvenile lobsters, *Homarus americanus* (Decapoda: Nephropidae), and comparison with the larval stages. *J. Crustac. Biol.* **12:** 467–510.

Martin, J. W., and B. W. Felgenhauer. 1986. Grooming behaviour and the morphology of grooming appendages in the endemic South American crab genus *Aegla* (Decapoda, Anomura, Aeglidea). *J. Zool. Lond.* **209:** 213–224.

Matsuura, H., and S. Nishida. 2000. Fine structure of the "button setae" in the deep-sea pelagic copepods of the genus *Euaugaptilus* (Calanoida: Augaptilidae). *Mar. Biol.* **137:** 339–345.

Nickell, L. A., J. A. Atkinson, and E. H. Pinn. 1998. Morphology of thalassinidean (Crustacea: Decapoda) mouthparts and pereiopods in relation to feeding, ecology and grooming. *J. Nat. Hist.* **32:** 733–761.

Paffenhöfer, G. A., and P. A. Loyd. 1999. Ultrastructure of the setae of the maxilliped of the marine planktonic copepod *Temora stylifera*. *Mar. Ecol. Prog. Ser.* **178:** 101–107.

Paffenhöfer, G. A., and P. A. Loyd. 2000. Ultrastructure of cephalic appendages setae of marine planktonic copepods. *Mar. Ecol. Prog. Ser.* **203:** 171–180.

Pinn, E. H., L. A. Nickell, A. Rogerson, and J. A. Atkinson. 1999. Comparison of the mouthpart setal fringes of seven species of mud-shrimps (Crustacea: Decapoda: Thalassinidea). *J. Nat. Hist.* **33:** 1461–1485.

Robberts, M. H. 1968. Functional morphology of the mouth parts of the hermit crab, *Pagurus longicarpus* and *Pagurus pollicaris*. *Chesapeake Sci.* **9:** 9–20.

Schembri, P. J. 1982. Functional morphology of the mouthparts and associated structures of *Pagurus rubricatus* (Crustacea: Decapoda: Anomura) with special reference to feeding and grooming. *Zoomorphology* **101:** 17–38.

Schmidt, M., and W. Gnatzy. 1984. Are the funnel-canal organs the 'campaniform sensilla' of the shore crab *Carcinus maenas* (Decapoda: Crustacea)? II. Ultrastructure. *Cell Tissue Res.* **237:** 81–97.

Steele, V. J., and D. H. Steele. 1999. Cellular organization and fine structure of type II microtrich sensilla in gammaridean amphipods (Crustacea). *Can. J. Zool.* **77:** 88–107.

Steinbrecht, R. A. 1997. Pore structure in insect olfatory sensilla: a review of data and concepts. *Int. J. Insect Morphol. Embryol.* **26:** 229–245.

Steinbrecht, R. A. 1998. Bimodal thermo- and hygrosensitive sensilla. Pp. 405–422 in *Microscopic Anatomy of Invertebrates, Insecta,* F. W. Harrison and M. E. Rice, eds. Wiley-Liss, New York.

Stemhuis, E. J., B. Dauwe, and J. J. Videler. 1998. How to bite the dust: morphology, motion pattern and function of the feeding appendages of the deposit-feeding thalassinid shrimp *Callianassa subterranea*. *Mar. Biol.* **132:** 43–58.

Steullet, P., and C. D. Derby. 1997. Coding of blend ratios of binary mixtures by olfactory neurons in the Florida spiny lobster, *Panulirus argus*. *J. Comp. Physiol. A* **180:** 123–135.

Steullet, P., H. S. Cate, and C. D. Derby. 2000a. A spatiotemporal wave of turnover and functional maturation of olfactory receptor neurons in the spiny lobster *Panulirus argus*. *J. Neurosci.* **20:** 3282–3294.

Steullet, P., H. S. Cate, W. C. Michel, and C. D. Derby. 2000b. Functional units of a compound nose: aesthetasc sensilla house similar populations of olfactory receptor neurons on the crustacean antennule. *J. Comp. Neurol.* **418:** 270–280.

Wiese, K. 1976. Mechanoreceptors for near-field water displacement in crayfish. *J. Neurophysiol.* **39:** 816–833.